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13. SUPPLEMENTARY NOTES

14. ABSTRACT The goals of the proposed studies are to: i) use imaging methods to non-invasively assess the temporal relationship between castration resistant prostate cancer (CRPC) growth, androgen receptor (AR) levels, angiogenesis, hypoxia, and translocator protein (TSPO) levels and ii) use imaging to temporally direct pathological examination of tissue in order to enhance the elucidation of mechanistic aspects of CRPC progression, specifically the invovlvement of HIF-1alpha and NF-kappaB, two pathways that increase AR activity during progression to CRPC. As described in the statement of work, the second year of this award focused on starting the acquisition of serial imaging data in the Pten / p53 double null mutant mouse model. Towards that end, we have successfully acquired anatomic MRI and PET data in orthotopic tumors within the Pten/p53 mouse model, to assess tumor volume, track growth and tumor angiogenesis. In fic speciregards to PET imaging, we have further characterized the use of FMISO, FDHT and TSPO imaging to evaluate tumor hypoxia, androgen receptor levels and translocator protein expression. The characterization of these imaging features has better informed their use in serial studies aiming to assess these biological features before and after castration.

15. SUBJECT TERMS
castration resistant prostate cancer, MRI, PET, FDHT, image optimization

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Introduction

In its advanced stages, prostate cancer (PCa) becomes clinically difficult to restrain due to failure of therapy and the development of castration resistant prostate cancer (CRPC). Thus, there is a compelling need to investigate the mechanisms leading to CRPC in order to develop more effective treatment strategies. The most common approach to biologically assess disease progression in mouse models of PCa is through pathological examination, which requires the sacrifice of mice at multiple arbitrary time points and, consequently, is unsuitable for the temporal characterization of physiological, cellular and molecular events leading to CRPC growth in a given animal. In recent years, however, there have been dramatic increases in the range and quality of information available from non-invasive imaging methods so that many potentially valuable imaging metrics are now available to quantitatively measure tumor growth, assess tumor status, and predict treatment response. To this end, our study aims to evaluate emerging, clinically-viable imaging metrics in an appropriate PCa animal model to serially assess tumor progression and establish which method (or combination of methods) is most accurate at predicting castration induced tumor regression and the subsequent recurrence of the castration resistant tumors. In particular, we proposed to non-invasively assess the temporal relationship between CRPC growth, androgen receptor (AR) levels, angiogenesis, hypoxia, cellular proliferation and apoptosis using Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET). Such studies could provide the scientific basis for the acceleration of these emerging imaging methods into clinical care and could have a direct impact on prostate cancer detection, staging and treatment monitoring. Additionally, we proposed to use multi-parametric imaging to temporally direct pathological examination of tissue in order to elucidate mechanistic aspects of CRPC progression, specifically the involvement of hypoxia (HIF-1α) and NF-κB, two essential pathways that increase AR activity during progression to CRPC. The proposed studies are being carried out in the genetically engineered *Pten/p53* conditional mouse model (Ptenpc-/-; *Trp53pc-/-* double-null mutants). Our preliminary studies revealed that prostate tumors in these mutant mice are initially sensitive to castration, as evidenced by tumor regression, but this is followed by tumor recurrence that is ultimately lethal. The regression response to castration and subsequent CRPC growth in these mice clinically recapitulate the disease progression observed in human prostate cancer undergoing androgen-ablation therapy (AAT). Therefore, this authentic mouse model provides a valuable and unique tool to study CRPC progression and dysregulated pathways that could be used as targets of novel therapeutic strategies. Our hypothesis is that multiparametric imaging of vascular, cellular and molecular events will identify stages that predict CRPC progression.

Body

The overall goal of the project in Year 2 was to utilize the imaging methods to track prostate tumor growth. In the Statement of Work, Tasks 7 – 9 describe the studies proposed in Year 2. These tasks along with the corresponding progress statements are described below.

Task 7: Acquire Serial PET FDHT and MRI datasets in second cohort of mice

Progress: In Year 1 we synthesized and acquired 16β-^{18}F-Fluoro-5α-Dihydrotestosterone (FDHT) data (in the first cohort of mice transferred to Vanderbilt from Meharry College), a tracer that targets androgen receptor (AR), in a small cohort of mice. In Year 2 we begin to characterize its use to dynamically track changes in androgen receptors (in the second cohort of mice transferred to Vanderbilt, n = 8). Unexpectedly, we have found that the uptake of FDHT in these orthotopic prostate tumors, prior to castration, is extremely variable across time and mice. Even in mice where tumors have grown quite large and are easily discernable by MRI the uptake of FDHT can be similar to or lower than background levels found in other tissues. Consequently, we have concluded that the use of FDHT to characterize androgen receptor levels, prior to castration, is unreliable. These findings are also consistent with recent reports in humans that FDHT uptake is low until men are treated with androgen ablation therapy, indicating that background AR levels limit FDHT uptake. This leads us to conclude that FDHT is only suitable for assessing AR levels following castration. As described below, in the next cohort of animals we will validate this by tracking FDHT uptake following castration.

Note that in all studies the following scan parameters were used (Varian 7T MRI system, TR = 2s, TE = 40ms, echo train length = 8, echo spacing = 10ms, matrix = 256 x 256, FOV = 25.6 x 25.6 mm, 20 slices, 1 mm slice thickness, number of excitations = 20, scan duration ~ 21 min). PET imaging was performed on a Concorde Microsystems microPET Focus 220. Approximately 120 uCi of tracer was administered via an indwelling jugular catheter and dynamic scans were acquired for 2 hours. CT data was acquired on an ImTek MicroCAT II in approximately eight minutes using the following imaging parameters: voltage of 80 kvp with an anode current of 500 μA, 360 projections in 1° steps, exposure time = 600 ms, acquisition matrix = 512^3.

Task 8: Transfer third cohort of Pten/p53 conditional double null mice to Vanderbilt (n = 10) and quarantine

Progress: Dr. Zhenbang Chen, Co-investigator at Meharry, has continued to maintain the the Pten/p53 conditional mice and was able to provide the mice needed for studies in Year 2. As he routinely uses these mice for other studies this colony is maintained and readily available for our studies going into Year 3. We did experience an unexpected delay in the transfer of these mice (which was supposed to occur in May / June of this year), however, due to a quarantine issue within Vanderbilt's Department of Animal Care. The animals could not be delivered until July and are currently nearing the end of their quarantine period. Consequently, this delayed to initiation of the serial

studies proposed in Task 9 until Oct 2014. This will not delay our studies proposed in Year 3 since it does not influence the transfer of the next cohort of animals.

Task 9: Acquire serial multi-modal and multi-parametric images

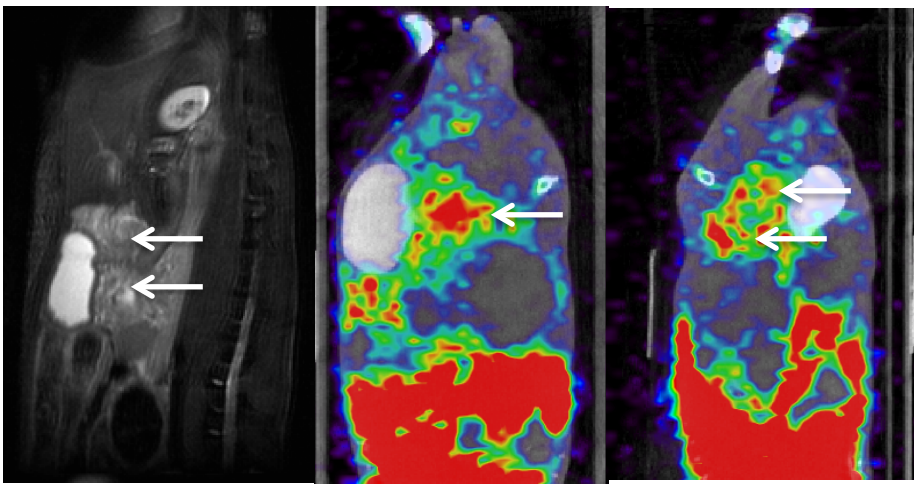

Figure 1: Representative MRI (left), and TSPO PET images (coronal slice center, transverse slice right) in a Pten/p53 mouse model.

Progress: As described in our last annual report one of our Year 2 goals was to next characterize the uptake of a PET tracer targeting translocator protein expression (TSPO), using 18F-VUIIS1008 (a probe developed in-house), and hypoxia, using 18F-Flouromisonidazole (FMISO) in Pten/p53 conditional mice (n = 8). These studies were conducted in the animals described in Task 7 (on days when FDHT data was not collected). In the same animals we acquired MRI, TSPO PET and FMISO PET over multiple time points. Interestingly, unlike FDHT the uptake of TSPO, pre-castration, was highly localized within the prostate tumors and, therefore, exhibited very high tumor to muscle ratios (>4 in most tumors). **Figure 1** shows representative MRI and TSPO PET images. Note the presence of tumor(s) near the bladder in both the MRI and PET images. **Figure 2** highlights the remarkable dynamic uptake of TSPO as compared to muscle. Across 60 minutes the %ID/cc continues to increase which is indicative of specific uptake and retention of the tracer. Another feature of this agent that makes it very attractive for prostate cancer imaging is that it shows no excretion via the bladder and very little uptake in the tissue

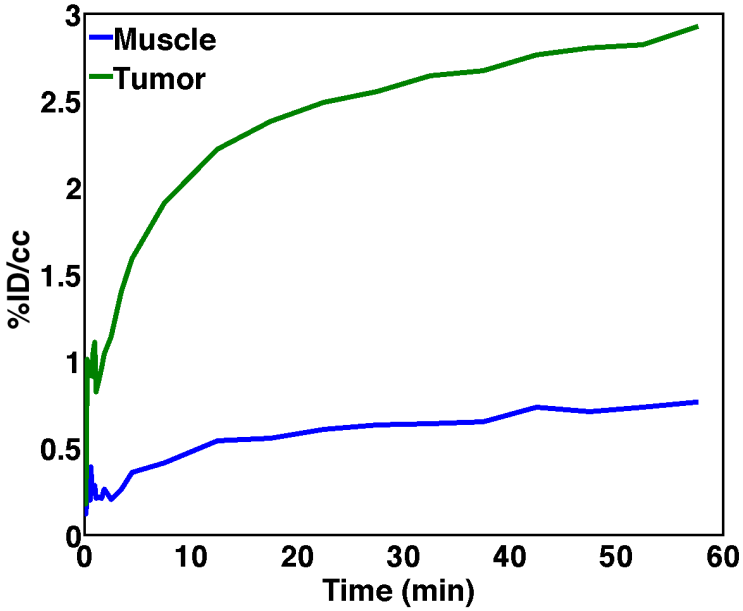

Figure 2: Representative dynamic PET TSPO data in Pten/p53 tumor and muscle regions of interest.

surrounding the prostate. The only other marked uptake was in liver which is also apparent in Figure 1. The accumulation of FMISO and FDHT within the bladder often times confounded the identification of small tumors that were in its vicinity (within a few mm) because its signal was as high as that found in tumors. Given the strong potential of TSPO for prostate cancer imaging we will perform a cold injection study following radiotracer injection to further verify specificity of the retained agent (along with histological validation of TSPO expression). These strong results warrant the careful validation of this tracer as it could lay the foundation for its use in humans.

The uptake of FMISO was more heterogeneous within and across tumors as compared to TSPO. **Figure 3** shows representative CT and FMISO PET in animals that were known to have considerable tumor burden as determined by MRI. In all animals the FMISO uptake in regions coinciding with the location of known tumors was quite similar to the surrounding tissue that would be expected to be normoxic. Even in the animal in the top row of Figure 4 the dynamic uptake of FMISO (shown in **Figure 4**) is not consistent with hypoxic tissue; exhibiting rapid and strong influx of tracer followed up efflux that is similar to surrounding tissue. In our other tumor models, the FMISO dynamics in hypoxic regions after its initial delivery slowly continues to increase indicating its reduction and cellular retention. Consequently, one way we identify hypoxic tissue using FMISO is to find voxels exhibiting positive slope following the initial delivery (e.g. between 30 – 60 minutes). Voxels with negative slope (such as that shown in Figure 4) would be considered normoxic. We expect histological validation (to be performed in the next cohort of animals) to show that these tumors are fairly normoxic.

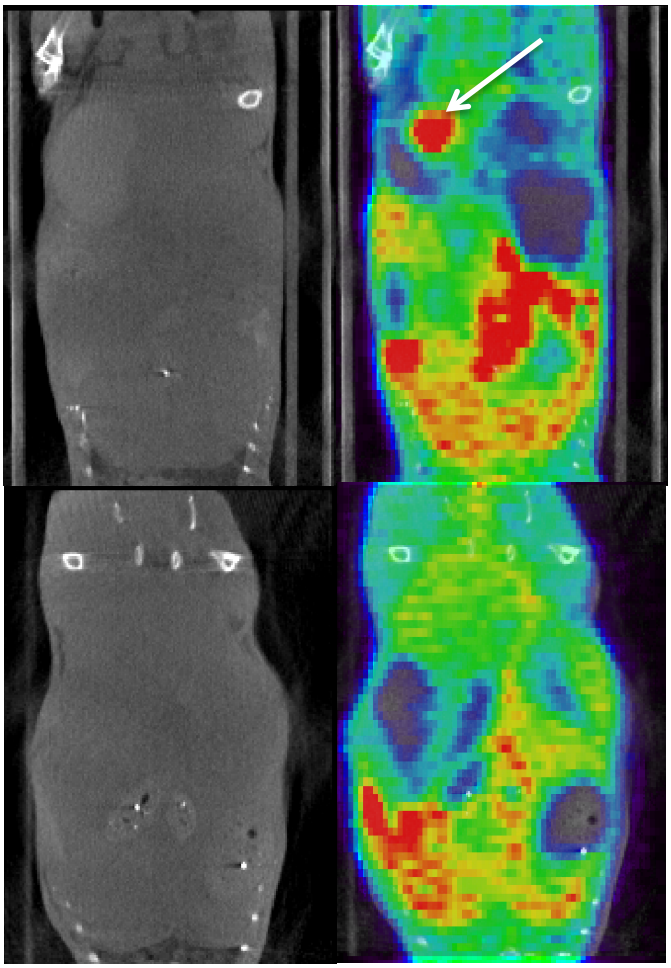

Figure 3: Representative FMISO PET images in two PTEN/p53 mouse with apparent tumor uptake (top) and no tumor specific uptake (bottom). Both animals had substantial tumor burden as determined by MRI.

Given these recent findings our plans for Year 3 will focus on the use of MRI (before and after castration), FDHT (following castration) and VUIIS-1008 (before and after castration) to track the regression and subsequent progression of tumors in the

Pten/p53 mouse model. As described in the proposal this data will be used to identify time points for IHC analysis.

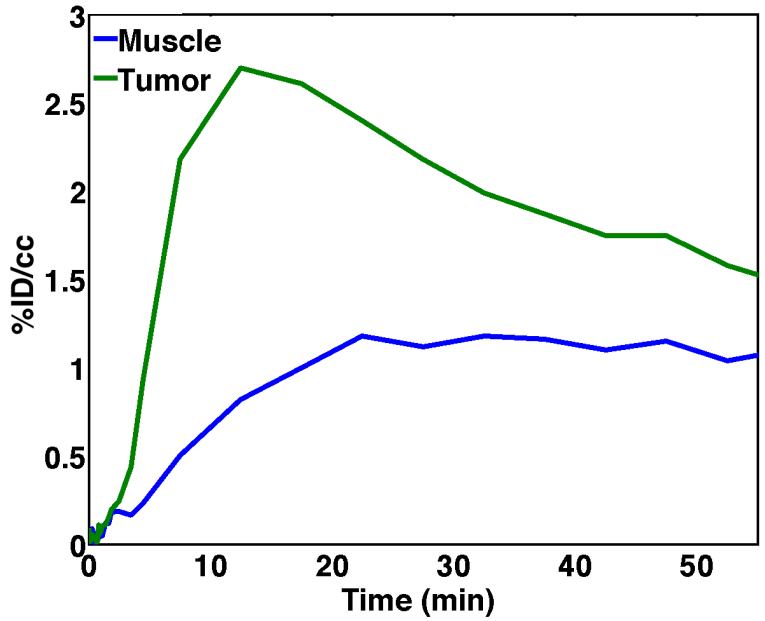

Figure 4: Representative dynamic PET FMISO data in Pten/p53 tumor and muscle regions of interest.

Personnel receiving pay from this research effort

C. Chad Quarles, Department of Radiology, Vanderbilt University (10% effort)
Dan Ayers, Department of Biostatistics, Vanderbilt University (5% effort)
Zhenbang Chen, Department of Biochemistry and Cancer, Meharry Medical College
Wenfu Lu, Department of Biochemistry and Cancer, Meharry Medical College

Key Research Accomplishments

- The use of high-resolution anatomic and contrast enhanced MRI methods to track tumor growth in Pten/p53 mouse models
- The first use and characterization of FMISO and TSPO PET compounds in the Pten/p53 mouse model
- This is the first study to demonstrate that TSPO uptake is highly localized in prostate cancer and, accordingly, could improve our ability to detect and track prostate cancer in humans as compared to conventional imaging methods.

Reportable Outcomes

Given that Year 2 was primarily focused on the continued characterization of molecular imaging agents and that these studies are ongoing we currently have nothing to report. We are currently in the process of preparing several manuscripts describing these efforts. First, we plan to submit a manuscript validating the use of the TSPO PET tracer developed in house in the Pten/p53 mouse model. Second, we plan to submit a manuscript comparing the uptake of TSPO, FDHT and FMISO tracers (in the same animals) before and after castration.

Conclusions

In Year 2 we have completed all of the elements on the approved Statement of Work except for imaging on one cohort of animals which will begin in Oct 2014. Perhaps the most noteworthy finding in this year is the marked uptake of VUIIS-1008 in prostate tumors as compared to the surrounding tissue. Unlike the other molecular imaging probes this agent shows uptake in all the tumors discernable by MRI and was consistent across mice. Going into year 3 the combination of our multimodal imaging approach will enable a more comprehensive and serial characterization of castration resistant prostate cancer progression. If successful, these efforts will also provide the scientific basis for the acceleration of these emerging methods into clinical care and could have a direct impact on prostate cancer detection, staging and treatment monitoring.